

Full Length Research Paper

Antifungal, acute toxicity and mutagenicity activity of extracts from *Datura stramonium*, *Jacquinia macrocarpa* and *Krameria erecta* on *Fusarium verticillioides*

M. P. Frías-Escalante, A. Burgos-Hernández, M. Plascencia-Jatomea, M. L. Aldana-Madrid and M. O. Cortez-Rocha*

Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Mexico.

Received 11 December, 2014; Accepted 13 July, 2015

The effect of *Baccharis glutinosa*, *Jacquinia macrocarpa*, and *Krameria erecta* extracts was investigated on the growth and the spore germination of *Fusarium verticillioides* (ATCC 52539). Brine shrimp (*Artemia salina*) was used to evaluate the potential acute toxicity of the fractions obtained from plant extracts. The butanol fraction of *J. macrocarpa* totally inhibited the radial growth for 144 h and up to 95% after 168 h. The ethyl acetate fraction of *B. glutinosa* caused 100% of radial growth inhibition for 96 h. The ethyl acetate fractions of *B. glutinosa* and *K. erecta* caused the higher inhibitory effect on *F. verticillioides* spore germination, 100 and 95%, respectively. All plant fractions tested at a concentration of 5.0 mg mL⁻¹ caused 100% brine shrimp lethality after 24 h. The Ames test did not reveal the presence of an evident mutagenic activity.

Key words: Antifungal activity, plant extracts, brine shrimp bioassay, mutagenicity assay, *Fusarium verticillioides*.

INTRODUCTION

The plant species in Mexico are more than 26,000 from which 4,000 are estimated to have medicinal use (Mittermeier and Goettsch, 1992). In addition, some of them have exhibited other properties such as antifungal activity and might be considered natural bioactive substances for the control of post-harvest fungal infections. Plant extracts are generally assumed to be more acceptable and less hazardous than synthetic

compounds and they might represent an alternative antifungal approach (Jobling, 2000).

Baccharis glutinosa Pers (syn.: *Baccharis salicifolia* (Ruiz & Pav.) Pers) and *Jacquinia macrocarpa* (syn.: *Jacquinia aurantica*), are traditional medicinal plants that belong to the *Asteraceae* and *Theophrastaceae* families, respectively (Barrows, 1967; Moreno-Salazar et al., 2008). Ethnic groups from Northwest Mexico have been

*Corresponding author. E-mail: mcortez@guayacan.uson.mx.

using *B. glutinosa* for gastrointestinal disorders whereas they have used *J. macrocarpa* to prepare a mustard-colored dye from the fruits and a tea out of the flowers that strengthens the heart (Yetman and Van Devender, 2002). Also, cytotoxic and anti-inflammatory properties have been reported for *B. glutinosa* extracts (Fukuda et al., 2006; Abad et al., 2006; Abad and Bermejo, 2007). These plants are widely distributed from Southwest U.S.A. to central Mexico (Barrows, 1967; Moreno-Salazar et al., 2008). *Jacquinia* is native from West Indies where it is known as *J. aurantica*. DiSalvo (1974) reported that *B. glutinosa* aqueous extract of dried powdered leaves to inhibit dermatophytes *in vitro*. He mentioned that *B. glutinosa* is recommended in the southwestern desert of the United States for the therapy of athlete's foot caused by *Tinea pedis*. In addition, *Datura stramonium* has been reported to have antifungal activity.

Fractions from methanolic extracts of these plants have shown antifungal properties against some phytopathogenic and toxigenic molds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* (Buitimea-Cantúa et al., 2013). Those authors reported radial growth inhibition, hyphal diameter and length, and mycotoxin production. Based on the above, the aim of this study was to evaluate the antifungal properties of fractions of extracts obtained from *Krameria erecta*, *Baccharis glutinosa*, and *J. macrocarpa* on *F. verticillioides*, and to evaluate their mutagenic potential and acute toxicity.

MATERIALS AND METHODS

Plant materials

J. macrocarpa Cav. spp. pungens and *Krameria erecta* Willd ex Schult were collected in the area of Los Arrieros, Sonora (Latitude N 28° 20.538' W 111° 08.911' altitude 280 feet and latitude N 28° 19.526' W 111° 08.828' altitude 227 feet) during August 2010. Aerial parts of *B. glutinosa* Pers. were collected during February 2011 in the riverside of Tecoripa River near the rural community of Tecoripa, Sonora. A voucher sample of each plant was deposited at the Herbarium of the Scientific Research and Technology Department of the University of Sonora (DICTUS) in Hermosillo, Sonora (Mexico) to confirm its identification. The plant specimens were sealed in plastic bags, and transported to the laboratory.

Preparation of antifungal extracts

Plants were sun dried (35–40°C) for 2 weeks and milled (Pulver 200, U.S.A.) to a particle size of 0.5–1.0 mm. Sixty grams of powdered aerial parts of each plant were extracted with 940 ml of 70% methanol by agitation for 1 h with a wrist action Burrel shaker (Burrel Corporation, Pittsburg, PA), and stored at 25°C for 3 days at darkness. The extracts were filtered first through Whatman filter paper No. 1 and then through micropore glass filter. The methanolic extracts (crude extracts) were evaporated to dryness at 40°C with vacuum in a Yamato rotary evaporator RE 300). Crude extracts were evaluated for antifungal activity. The extracts that showed the highest inhibition activity were evaporated to dryness and subjected to fractionation. Twenty grams of dried extracts were suspended in

1 L of water and sequentially partitioned with hexane, ethyl acetate, and n-butanol (Koketsu et al., 1996) and all were tested for antifungal activity. Plates with potato dextrose agar medium, PDA, (DIFCO, USA) were prepared using 5 mg mL⁻¹ of each fraction [Ethyl acetate fraction of *B. glutinosa* and *K. erecta* (FAe Bg) and (FAe Ke), respectively and n-butanol fraction of *J. macrocarpa* (FB Jm)]. Petri dishes containing PDA prepared with each of the different solvents used for fractionation were used as controls.

Antifungal activity assay

A strain of *F. verticillioides* (ATCC 52539) was selected for its high fumonisin production. Fungal strain was activated in PDA and incubated at 25 ± 2°C for 10 days. Spores were harvested by pouring a sterile solution of 0.1% (v/v) Tween 80 into the flask and stirring the suspension with a sterile magnetic bar for 5 min. Spore concentration of the suspension was determined using a Neubauer chamber and adjusted to a final concentration of 1 × 10⁵ spores/mL. Petri dishes containing PDA medium prepared with 5 mg mL⁻¹ of plant extract fractions were centrally point-inoculated with 1 × 10⁵ spores/mL and incubated in the darkness at 25°C for 7 days. Two types of controls were prepared, one contained PDA medium plus aliquots of each solvent and the other one containing only PDA media. Colony diameters were measured every 24 h using a caliper and compared to those grown in the control media until the fungal growth in the control reached the plate border. All the measurements were carried out by triplicate. The radial growth inhibition percentage was calculated using the following equation: Radial Inhibition (%) = [(Rc-Ri)/Rc] × 100. Where Rc is the mean value of the colony radius in the control media and Ri is the colony radius value of the colonies grown in PDA amended with the partitioned extracts.

Germination of spores

Petri dishes containing PDA amended with 5 mg mL⁻¹ of extract fractions (FAe Bg, FAe Ke, and FB Jm) were inoculated by spreading 3 µl of a spore suspension containing 1 × 10⁴ spores/mL and incubated at 25°C using a 12 h light/dark cycle (Precision Low temperature Illuminated Incubator 818, U.S.A.). Two types of controls were prepared, one contained PDA medium plus aliquots of each solvent and the other one contained only PDA media. Samples were taken every 4 h of incubation time and 200 spores were counted at random (germinated and non-germinated) using light microscope. Count of spores was performed until the control reached 100% of spores germinated. The number of germinated spores per plate was determined. A spore was considered germinated when the length of its germinal tube reached one-half of the spore diameter. Each germination experiment was made by triplicate. The inhibition of spore germination was determined using Equation 1, in which Si represents the percentage of germinated spores in the plates treated with the extract fraction, and Sc was the percentage of germinated spores in the control containing each of the solvents (Paul et al., 1993).

$$\text{Inhibition (\%)} = \frac{\%S_c - \%S_t}{\%S_c} \times 100$$

Brine shrimp bioassay

In order to evaluate the potential acute toxicity of the fractions obtained from plant extracts, brine shrimp larvae assay was used (Jiménez et al., 1997). Dried *Artemia salina* eggs (0.1 g) were

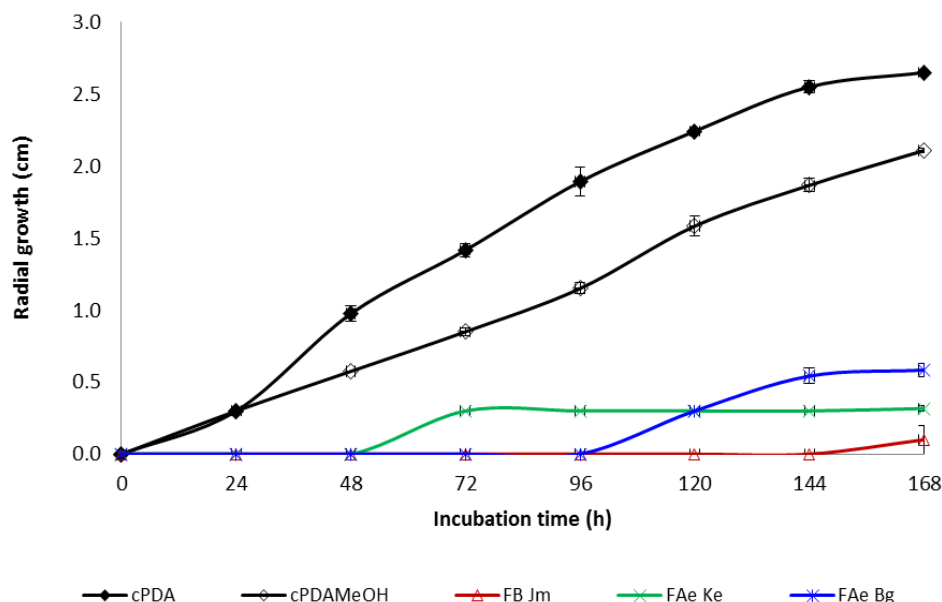


Figure 1. Radial growth of *Fusarium verticillioides* in PDA amended with 5 mg mL⁻¹ of extracts fractions from *Jacquinia macrocarpa* (FB Jm), *Krameria erecta* (FAe Ke), and *Baccharis glutinosa* (FAe Bg).

deposited in 1 L of sterile marine water with aeration and light during 24 h to hatch. Brine shrimp larvae were exposed to 5.0, 0.5, 0.005, and 0.0005 mg mL⁻¹ of the extract fractions for 24, 48, and 72 h. The number of dead larvae was recorded every 24 h to estimate the percentage of mortality. The assay was carried out by quintuplicate.

Mutagenicity assay

The mutagenic potential of the fractions obtained from plant extracts was determined according to the procedure described by Maron and Ames (1983) using *Salmonella* tester strains TA98 and TA100, with and without bioactivation (S9). Each was placed on nutrient broth (Difco Nutrient Broth) for reproduction during 12 h at 37°C in a circulation water bath at dark. One-hundred microliters of partitioned plant extracts, FAe Bg, FAe Ke and FB Jm, were deposited in test tubes (5, 0.5, 0.005, 0.0005, 0.00005, and 0.00005 mg mL⁻¹). Then, each tube was combined with 2.0 mL of bacteriologic agar (Sigma Chemical Co.) supplemented with histidin and biotin, 100 µL of bacterial culture, and 500 µL of S9 mix. This mixture was poured onto minimal glucose agar Petri dishes and incubated for 48 h at 37°C. For mutagenicity, positive control sodium azide was used (without S9) and aflatoxin B1 (with S9). The number of revertants was counted using a colony counter and compared against the controls. The assay was carried out by triplicate.

A completely randomized design of the radial growth and spore germination was carried out. The JMP 2004 software computed the analysis of variance and the means were compared with the Tukey multiple range tests ($P < 0.05$) (JMP vs. 5.0, SAS Institute Inc., USA).

RESULTS AND DISCUSSION

The extract fractions exhibited a moderate to high

antifungal activity against *F. verticillioides*. No fungicide effect was observed, only an inhibitory activity was detected in the fungus growth when compared to controls. Controls with solvents and pure PDA control showed the higher radial growth. When inoculated on PDA containing the extracts fractions, the radial growth of the fungi was delayed during the incubation time (Figure 1). The FB Jm totally inhibited the radial growth for 144 h and up to 95% after 168 h (Table 1). On the other hand, the FAe Bg caused 100% of radial growth inhibition for 96 h, after that the inhibitory effect was reduced to 72%. Treatment with FAe Ke also inhibited the radial growth in 100% for 48 h and 65% after 72 h of incubation. These results are in agreements with a previous work (Rosas-Burgos et al., 2009), which reported an inhibition of 67% of *F. verticillioides* radial growth. This result is of relevance for the present study because it confirms that *Baccharis glutinosa* has a fungistatic activity; plant specimens used in the present study were collected in a different year to those used by Rosas-Burgos et al. (2009) and similar results were reached. This might suggest that bioactive compounds are present in *B. glutinosa* independently of the year in which the plant is collected. Nevertheless, a recent study showed that concentration of total phenolic compounds and flavonoids on *B. dentata* showed significant seasonal variation (Sartor et al., 2013). Regarding the chemical constituents found in the genus, coumarins, flavonoids and terpenoids are the most frequently reported (Cifuentes et al., 2002; Simoes-Pires et al., 2005; Wachter et al., 1999). On other hand, Kurdela et al. (2010) isolated three coumarins from *Baccharis darwinii*. The effect of extracts may be

Table 1. Radial growth inhibition (%) of *Fusarium verticillioides* in PDA amended with 5 mg mL⁻¹ of extracts fractions from *Jacquinia macrocarpa* (FB *Jm*), *Krameria erecta* (FAe *Ke*), and *Baccharis glutinosa* (FAe *Bg*).

Incubation time (h)	FB <i>Jm</i>	FAe <i>Ke</i>	FAe <i>Bg</i>
48	100 ± 0.0	100 ± 0.0	100 ± 0.0
72	100 ± 0.0	65 ± 0.0	100 ± 0.0
96	100 ± 0.0	74 ± 0.0	100 ± 0.0
120	100 ± 0.0	81 ± 0.0	81 ± 0.0
144	100 ± 0.0	84 ± 0.0	71 ± 5.1
168	95 ± 8.2	85 ± 1.4	72 ± 3.6

FB *Jm* = n-Butanol fraction of *J. macrocarpa*; FAe *Ke* = ethyl acetate fraction of *K. erecta*; FAe *Bg* = ethyl acetate fraction of *B. glutinosa*.

due to their chemical composition and probably to the membrane composition of the fungi. *B. dracunculifolia* DC, a native plant of South America, is one of the most studied of this genus and baccharin (3-prenyl-4-(dihydro-cinnamoyloxy) cinnamic acid) is the chemical compound isolated from its aerial parts. Tabti et al. (2014) mentioned that terpene hydrocarbons and phenolic compounds affects the fungi development. Information on the mechanism(s) of action by these type of compounds in *Baccharis* is not available. Velluti et al. (2005) mentioned that other authors have attributed it not only to the presence of terpenes, phenolic compounds, and other components, but also to the chemical structure, such as the presence of hydroxyl groups in their phenolic compounds.

Also, the values of radial extension rate, determined from the slope of the radial growth versus time during the linear growth phase, were reduced (Table 2). The lower value corresponds to the treatment with FB *Jm* which caused 100% of inhibition. Result indicate that spores inoculated on control treatments began to germinate after 4 h and reached the 100% of germination at 14 h. Spores inoculated on media containing FAe *Bg* and FAe *Ke* caused 100 and 95% germination, respectively at 14 h. On the other hand, spores cultivated in the presence of FB *Jm* were poorly affected.

The fractions FAe *Bg* and FAe *Ke* caused higher inhibitory effect on *F. verticillioides* spore germination, 100 and 95%, respectively (Figure 2). FB *Jm* exerted the lowest effect on spore germination inhibiting only 19.0% and allowed the higher germination velocity compared to the other plant fractions (Figure 2). The first morphological change in spore germination is called swelling in which the diameter of the spore increases. It involves water uptake and a decrease in the microviscosity of the cytoplasm. Also, molecules are directed to the cell cortex to enable addition of new plasma membrane and cell wall (Bartnicki-Garcia and Lippman, 1977; Momany, 2002). At later stages of development, the growth speed of the germ tube increases and the

Table 2. *Fusarium verticillioides* spore germination rate on PDA with and without the evaluated extracts fraction (5 mg mL⁻¹).

Treatment	Spore germination rate (% EG h ⁻¹)	Radial extension rate (cm h ⁻¹)
FAe <i>Bg</i>	0.000	0.0113
FAe <i>Ke</i>	0.354	0.0125
FB <i>Jm</i>	1.438	0.0003
cPDAMeOH	11.406	0.0129
cPDA	11.750	0.0185

FAe *Bg* = *Baccharis glutinosa* ethyl acetate fraction; FAe *Ke* = *Krameria erecta* ethyl acetate fraction; FB *Jm* = *Jacquinia macrocarpa* n-butanol fraction; cPDAMeOH = PDA plus methanol control; cPDA = Control of PDA; EG h⁻¹ = Germinated spores.

functional organization of the hyphal tip area acquires its full potential. The structure of the fungal cell wall is unique to the fungi and it is composed of chitin, glucans, mannans and glycoproteins (Bowman and Free, 2006). Damage on the fungal cell wall produces morphological alterations, inhibition of fungal growth or apoptotic cell death (Escalante et al., 2008; Alonso et al., 2010; Khan and Nasreen, 2010), which are presumably the result of alterations caused to the components of the cell wall, β -glucan and chitin. Recent research has shown that extracts from *B. glutinosa* and *J. macrocarpa* have chitinase activity against polymeric extracts from *A. flavus* and *F. verticillioides* (Buitimea-Cantúa et al., 2013), which helps to explain our findings.

Table 3 shows the data obtained from the negative control of *A. salina* suspended in marine water. *A. salina* exposed to sodium azide (5.0 and 0.5 mg mL⁻¹, positive control) reached 100% mortality after 24 h. All plant fractions tested at a concentration of 5.0 mg mL⁻¹ caused 100% brine shrimp lethality after 24 h. The extract fraction from *J. macrocarpa* (BF *Jm*) showed similar toxicity effects than sodium azide after 24 h when *A. salina* was either exposed to 5.0 or 0.5 mg mL⁻¹. However, when BF *Jm* concentration decreased, the brine shrimp mortality also diminished. This plant extract fraction caused the lowest mortality from the three plant extracts evaluated. The other two extracts showed similar mortality at all of the concentrations evaluated. Our results suggest the presence of toxic compounds in each of the plant extracts, which are able to physiologically affect *A. salina*.

The positive mutagenicity controls, sodium azide and aflatoxin B1, tested in *Salmonella* Thyphimurim strains TA 98 and TA 100, are presented in Table 4. We observed that they were sensitive in this experiment and reproducible results could be achieved. Plant extracts did not induced any mutagenic effect on both *Salmonella* tester strains (Table 5). Mutagenicity exerted by extracts was considered negative since the number of revertant

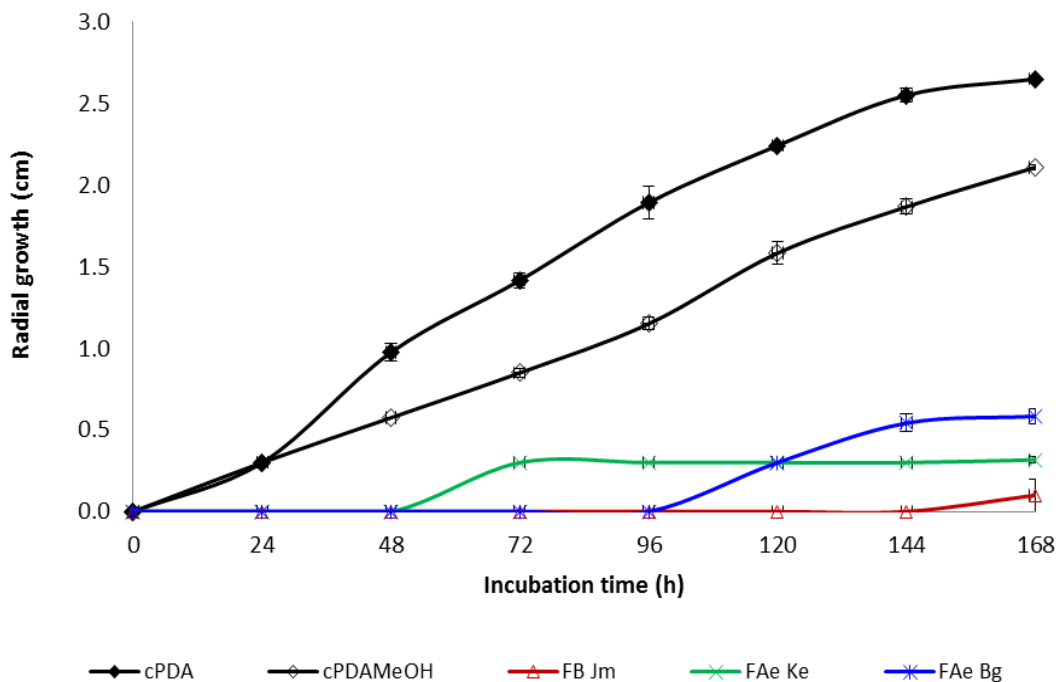


Figure 2. Radial growth of *Fusarium verticillioides* in PDA amended with 5 mg mL⁻¹ of extracts fractions from *Jacquinia macrocarpa* (FB Jm), *Krameria erecta* (FAe Ke), and *Baccharis glutinosa* (FAe Bg).

Table 3. Mortality of *Artemia salina* when exposed to different treatments

Treatment (mg mL ⁻¹)	Exposition time (h)		
	24	48	72
Marine water with sodium azide	1.67 ± 1.7 ^a	4.71 ± 4.7 ^a	84 ± 5 ^a
0.005	4 ± 4 ^a	36 ± 12 ^b	84 ± 2 ^a
0.05	24 ± 6 ^b	58 ± 13 ^c	93 ± 5 ^b
0.5	100 ± 0 ^c	100 ± 0 ^f	100 ± 0 ^c
5.0	100 ± 0 ^c	100 ± 0 ^f	100 ± 0 ^c
FB Jmb			
0.005	26 ± 4 ^b	55.6 ± 12 ^{bc}	92.71 ± 3 ^b
0.05	55 ± 10 ^c	70 ± 10 ^d	97 ± 2 ^b
0.5	99 ± 0.8 ^e	100 ± 0 ^f	100 ± 0 ^c
5.0	100 ± 0 ^e	100 ± 0 ^f	100 ± 0 ^c
FAe Ke			
0.005	54.7 ± 10 ^c	86 ± 5 ^e	97 ± 2 ^b
0.05	88 ± 8.5 ^d	96 ± 4 ^f	100 ± 0 ^c
0.5	81.1 ± 6.8 ^d	100 ± 0 ^f	100 ± 0 ^c
5.0	100 ± 0 ^e	100 ± 0 ^f	100 ± 0
FAe Bg			
0.005	49.1 ± 14 ^c	89 ± 4.6 ^e	99.2 ± 0.8 ^c
0.05	60.2 ± 12 ^c	95.5 ± 2 ^{ef}	100 ± 0 ^c
0.5	87.5 ± 5 ^d	92.8 ± 3 ^f	100 ± 0 ^c
5.0	100 ± 0 ^e	100 ± 0 ^f	100 ± 0 ^c

FB Jm = *Jacquinia macrocarpa* n-butanol fraction; FAe Ke = *Krameria erecta* ethyl acetate fraction; FAe Bg = *Baccharis glutinosa* ethyl acetate fraction.

Table 4. *Salmonella* test strains TA98 and TA100, with and without bioactivation (S9) exposed to different concentrations of mutagenicity positive controls.

Concentration (mg mL ⁻¹)	TA 98			TA 100		
	RI	RE	RM	RI	RE	RM
Sodium azide without S9						
0.015	922 ± 65 ^b	26	35	2140 ± 111.4	224	10
0.15	1147 ± 117 ^c	26	44	2388 ± 67.7	224	11
1.5	2053 ± 88 ^d	26	79	3474 ± 317.0	224	15
15	253 ± 42 ^a	26	10	1626 ± 213.0	224	7
Aflatoxin B1 with S9						
5	41.0 ± 7 ^a	26	1.58	236 ± 12.5	224	1.16
50	42.0 ± 3 ^a	26	1.63	278 ± 15.6	224	1.36
500	932.0 ± 46 ^c	26	35.86	2867 ± 415.0	224	14.1
5000	205.0 ± 9 ^b	26	7.88	329 ± 66.3	224	1.6

RE = Spontaneous revertants; RI = induced revertants; RM = mutagenicity ratio.

Table 5. Antimutagenic potential of the plant extracts fractions. Both *Salmonella* test strains TA98 and TA100, with and without bioactivation (S9), were exposed to different concentrations of the extracts fractions.

Concentration (mg mL ⁻¹)	Bg FAe TA 100	Bg FAe TA 98	Jm FB TA 100	Jm FB TA 98	Ke FAe TA 100	Ke FAe TA 98
With S9						
0	231.5 ± 10.6	34.5 ± 12.0	162.5 ± 48.8	47.5 ± 9.2	187 ± 83.4	45.5 ± 12.0
0.00005	201.5 ± 17.7	33 ± 4.2	229 ± 82.3	49 ± 11.3	272.5 ± 13.4	36 ± 5.7
0.0005	217 ± 42.4	44.5 ± 12.0	221 ± 77.8	53 ± 8.5	276 ± 9.9	36 ± 0
0.005	271.5 ± 10.6	40 ± 4.2	258.5 ± 51.6	43 ± 4.2	261.5 ± 37.5	34.5 ± 4.9
0.05	245.5 ± 7.8	36 ± 4.2	257.5 ± 21.9	34 ± 1.4	271.5 ± 50.2	30.5 ± 6.4
0.5	241.5 ± 24.7	38 ± 1.4	277 ± 15.6	38.5 ± 7.8	295 ± 46.7	53 ± 29.7
5	191 ± 48.1	41.5 ± 2.1	278.5 ± 16.3	42 ± 21.2	210 ± 53.7	34 ± 41.0
Without S9						
0	231.5 ± 10.6	34.5 ± 12.0	162.5 ± 48.8	47.5 ± 9.2	187 ± 83.4	45.5 ± 1.0
0.00005	212 ± 4.2	35 ± 14.1	242 ± 15.6	43.5 ± 10.6	225 ± 2.8	40 ± 0
0.0005	214 ± 5.7	32 ± 5.6	248 ± 26.8	37 ± 1.4	256 ± 19.8	39 ± 9.8
0.005	225.5 ± 0.7	28 ± 0	237.5 ± 16.7	41 ± 1.4	194 ± 39.6	36 ± 9.8
0.05	250.5 ± 36.6	26 ± 2.8	240.5 ± 0.7	38 ± 4.24	204 ± 42.4	42 ± 19.8
0.5	231.5 ± 3.5	26.5 ± 0.7	242 ± 1.4	42 ± 0	229.5 ± 24.7	44.5 ± 9.2
5	192 ± 11.3	28.5 ± 3.5	266 ± 7.1	33 ± 1.4	230.5 ± 24.7	56 ± 12.7

All values represent mean of triplicate determination ± standard deviation.

per plate observed did not double the number of spontaneous revertants. Similar findings have been reported (Nogueira et al., 2011); they found that fractions from *Baccharis trimera*, evaluated *in vivo* and *in vitro*, were not mutagenic. Also, other authors reported no genotoxic activity of *Baccharis incarum* on *Drosophila melanogaster* (Berzain and Rodrigo, 2006). These findings suggest that the genus *Baccharis* might not be of potential toxicity to superior animal organisms; however, further investigation should be performed for a full toxicity assessment.

This study indicates that the plant extracts had anti-fungal activity on *F. verticillioides* and can be exploited in the future to reduce fungal spread. They delayed radial growth during the incubation time. Ethyl acetate fractions of *B. glutinosa* and *K. erecta* caused the higher inhibitory effect on *F. verticillioides* spore germination, 100 and 95%, respectively. Suppression on spore production could be the major contribution to limit the pathogen spread. Plant fractions tested at 5.0 mg mL⁻¹ caused 100% brine shrimp lethality after 24 h and the Ames test did not reveal mutagenic activity.

Conflict of interests

The author(s) did not declare any conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to CONACYT (Mexican Council for Science and Technology) for the grant 58249.

REFERENCES

- Abad MJ, Bermejo P (2007). *Baccharis* (Compositae): A review update. *Arkivoc*. 7:76-79.
- Abad MJ, Bessa AL, Ballarin B, Aragon O, Gonzalez E, Bermejo P (2006). Anti-inflammatory activity of four Bolivian *Baccharis* species (Compositae). *J. Ethnopharmacol.* 103(3):338-244.
- Alonso F, Cirigliano AM, Cabrera GM, Ramírez JM (2010). Synthesis and preliminary biological screening of sterol analogues as new antifungal agents against plant pathogens. *Steroids* 75(10): 659-664.
- Barrows DP (1967). *Ethno-botany of the Coahuilla Indians of Southern California*. Banning, CA: Malki Museum Press. p. 78.
- Bartnicki-Garcia S, Lippman E (1977). Polarization of cell wall synthesis during spore germination of *Mucor rouxi*. *Exp. Mycol.* 1(3): 230-240.
- Berzain C, Rodrigo G (2006). Evaluación genotóxica del extracto etéreo de *Baccharis incarum*. *BIOFARBO* 14:11-15. <http://www.ops.org.bo/textocompleto/rmbiofa20061402.pdf>
- Bowman SM, Free SJ (2006). The structure and synthesis of the fungal cell wall. *Bioessays* 28(8):799-808.
- Buitimea-Cantúa G, Rosas-Burgos E, Cinco-Moroyoqui F, Burgos-Hernández A, Plascencia-Jatomea M, Cortez-Rocha M, Gálvez-Ruiz J (2013). *In vitro* effect of antifungal fractions from the plants *Baccharis glutinosa* and *Jacquinia macrocarpa* on chitin and β -1,3-glucan hydrolysis of maize phytopathogenic fungi and on the fungal β -1,3-glucanase and chitinase activities. *J. Food Saf.* 33(4):526-535.
- Cifuentes DA, Borkowski EJ, Sosa ME, Gianello JC, Giordano OS, Tonn CE (2002). Clerodane diterpenes from *Baccharis sagittalis*: insect antifeedant activity. *Phytochemistry* 61(8): 899-905.
- DiSalvo AF (1974). Antifungal properties of a plant extract I. Source and spectrum of antimicrobial activity. *Mycophatol. Mycol. Appl.* 54(2): 215-219.
- Escalante A, Gattuso M, Pérez P, Zacchino S (2008). Evidence for the mechanism of action of the antifungal phytolaccoside B isolated from *Phytolacca tetramera* Hauman. *J. Nat. Prod.* 71(10):1720-1725.
- Fukuda M, Ohkoshi E, Makino M, Fujimoto Y (2006). Studies on the constituents of the leaves of *Baccharis dracunculifolia* (Asteraceae) and their cytotoxic activity. *Chem. Pharm. Bull.* 54(10): 1465-1468.
- Jiménez M, Huerta T, Mateo R (1997). Mycotoxin production by *Fusarium* species isolated from bananas. *Appl. Environ. Microbiol.* 63(2):364-369.
- Jobling J (2000). Essential oils: A new idea for postharvest disease control. *Good Fruit and Vegetables Magazine* 11: 50.
- Khan ZS, Nasreen S (2010). Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. *J. Agric. Technol.* 6(4):793-805.
- Koketsu M, Kim M, Yamamoto T (1996). Antifungal activity against food-borne fungi of *Aspidistra eliator* Blume. *J. Agric. Food Chem.* 44(1):301-303.
- Kurdela RR, Lima B, Tapia A, Egly Feresin G, Gonzalez Sierra M, Rodríguez MV, Zacchino S, Enriz RD, Freile ML (2010). Antifungal activity of extracts and prenylated coumarins isolated from *Baccharis darwinii* Hook & Arn. (Asteraceae). *Molecules* 15(7):4898-4907.
- Maron D, Ames B (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* 113(3-4):173-215.
- Mittermeier R, Goettsch C (1992). La importancia de la diversidad biológica de México. In: *México ante los Retos de la Biodiversidad*. Edited by Comisión Nacional para la Biodiversidad. México, D.F. pp. 57-62.
- Momany M (2002). Polarity in filamentous fungi: Establishment, maintenance and new axes. *Curr. Opin. Microbiol.* 5(6): 580-585.
- Moreno-Salazar SF, Robles-Zepeda RE, Johnson DE (2008). Plant folk medicines for gastrointestinal disorders among the main tribes of Sonora, Mexico. *Fitoterapia* 79(2):132-141.
- Nogueira NPA, Reis PA, Laranja GAT, Pinto AC, Aiub CAF, Felzenszwalb I, Paes MC, Bastos FF, Bastos VLFC, Sabino KCC, Coelho MGP (2011). *In vitro* and *in vivo* toxicological evaluation of extract and fractions from *Baccharis trimera* with anti-inflammatory activity. *J. Ethnopharmacol.* 138(2):513-522.
- Paul GC, Kent CA, Thomas CR (1993). Viability testing and characterization of germination of fungal spores by automatic image analysis. *Biotechnol. Bioeng.* 42(1):11-23.
- Rosas-Burgos EC, Cortez-Rocha MO, Cinco-Moroyoqui FJ, Robles-Zepeda RE, López-Cervantes J, Sánchez-Machado DI, Lares-Villa F (2009). Antifungal activity *in vitro* of *Baccharis glutinosa* and *Ambrosia confertiflora* extracts on *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides*. *World J. Microb. Biotechnol.* 25(12): 2257-2261.
- Sartor T, Xavier MA, Mondin CA, dos Santos MA, Cassel A, Astarita LV, Satarém ER (2013). Seasonal changes in phenolic compounds and in the biological activities of *Baccharis dentata* (Vell) G.M. Barroso. *Ind. Crop Prod.* 51: 355-359.
- Simoës-Pires CA, Queiroz EF, Henriques AT, Hostettmann K (2005). Isolation and on-line identification of antioxidant compounds from three *Baccharis* species by HPLC-UV-MS/MS with post-column derivatisation. *Phytochem. Anal.* 16(5):307-314.
- Tabti L, Dib MEA, Gaouar N, Samira B, Tabti B (2014). Antioxidant and antifungal activity of extracts of the aerial parts of *Thymus capitatus* (L.) Hoffmanns against four phytopathogenic fungi of *Citrus sinensis*. *Jundishapur J. Nat Pharm. Prod.* 9(1):49-54.
- Velluti A, Sanchis V, Ramos AJ, Egido J, Marin S (2005). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. *Int. J. Food Microbiol.* 89(2-3):145-154.
- Wachter GA, Montenegro G, Timmermann B (1999). Diterpenoids from *Baccharis pingraea*. *J. Nat. Prod.* 62(2):307-308.
- Yetman D, Van Devender T (2002). Mayo ethnobotany: Land, history, and traditional knowledge in Northwest Mexico. University of California Press, 372 p.